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(54) Title: INHIBITORS OF FACTOR XII ACTIVATION AND APPLICATIONS THEREOF

(57) Abstract

Compositions and methods for prophylactically or therapeutically treating sepsis consisting of an inhibitor of Factor XII activation wherein the inhibitor is preferably antibody and has the exemplary properties: binds to and inactivates Factor XII in plasma; substantially inhibits the enzymatic activity of Factor XIIa; and is substantially unreactive with Factor XII-C1 complexes.

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INHIBITORS OF FACTOR XII ACTIVATION AND APPLICATIONS THEREOF

This invention is in the area of immunology/biochemistry, and presents inhibitors of Factor XIIa activation and medical applications of the inhibitor, alone or in combination, for the prophylactic or therapeutic treatment of sepsis, and the prevention of toxic side effects associated with cytokine therapy. The preferred inhibitors are antibody which may include polyclonal, or monoclonal antibody, or fragments derived therefrom, or recombinant constructs having the binding activity of such antibodies.

In the United States alone nosocomial bacteremia develops in about 194,000 patients, and of these about 75,000 die. Maki, D.G., 1981, Nosocomial Infect., (Dikson, R.E., Ed.), page 183, Yrke Medical Books, U.S.A.. Most of these deaths are attributable to six major gram-negative bacilli, and these are Pseudomonas aeruginosa, Escherichia coli, Proteus, Klebsiella, Enterobacter and Serratia. The current treatment for bacteremia is the administration of antibiotics which, unfortunately, have limited effectiveness.

Although the precise pathology of bacteremia is not completely elucidated, it is believed that bacterial endotoxins, lipopolysaccharides (LPS), are the primary causative agents. LPS consist of at least three significant antigenic regions, the lipid A, core polysaccharide, and O-specific polysaccharide. The latter is also referred to as O-specific chain or simply O-antigen. The O-specific chain region is a long-chain polysaccharide built up from repeating polysaccharide units. The number of polysaccharide units differs among different bacterial species and may vary from one to as many as six or seven monosaccharide units. While the O-specific chain varies among different gram-negative bacteria, the lipid A and core polysaccharides are similar if not identical. Since LPS plays a key role in sepsis, a variety of approaches have been pursued to neutralize its activity. Presently, there is considerable work which suggest that antibody to LPS will soon be a valuable clinical adjunct to the standard antibiotic therapy.

LPS initiates a cascade of biochemical events that eventually causes the death of the patient. One of the clinical symptoms of sepsis is intravascular coagulation which is reflected in decreased plasma concentrations of various coagulation factors, such as Factor XII. This aspect of the clinical course of the disease is consistent with in vitro studies which have shown that LPS can activate both the contact system of intrinsic coagulation, as well as the complement system. Morris, E.C., et al., 1974, J. of Experimental Med., 140:797 and Morrison, D.C., et al., 1978, American Journal of Pathology, 93:527.

Factor XII, also known as Hageman Factor, is a plasma serine protease zymogen. Mason, J.W., et al., 1979, Annals of Internal Medicine, 73:545. Human Factor XII is a single-chain Beta globulin of molecular weight 80,000. During activation, it is cleaved to a two-chain enzyme, termed Factor XIIa, consisting of a heavy chain of 50,000 molecular weight and a light chain of 28,000 molecular weight. Both chains are linked together by a single disulfide bridge. The light chain is enzymatically active, while the heavy chain binds to solid surfaces during contact activation.

LPS leads to the activation of Factor XII, which in turn is involved in causing coagulation, a major clinical manifestation of sepsis. In addition, activation of Factor XII is involved in causing hypotensive reactions via activation of prekallikrein, which in turn cleaves bradykinin from kininogen. Colman, 1984, J. Clin. Invest. 73:1249. Thus, it will appreciated that agents that prevent the activation of Factor XII would be valuable therapeutics or prophylactics that could be used to prevent or treat disease.

Both monoclonal and polyclonal antibody to Factor XII have been described. For example, monoclonal antibody that binds to the heavy chain of Factor XII is described by Small et al., (1985) Blood, vol. 65: p. 202; Saito et al., (1985) Blood, vol. 65: p. 1263; and Pixley et al., (1987) J. Biol. Chem., vol. 262: 10140. Monoclonal antibody to the light chain of Factor XII are shown in PCT patent application WO 89/11865.

Polyclonal antibody has been reported by Lammle et al., (1986) Anal. Biochem. vol. 156: p.118 and Lammle et al., (1986) Thromb. Res. vol. 41: p. 747.

A first object of the invention is a description of inhibitors of Factor XII activation that are useful medicaments for the prophylactic or therapeutic treatment of sepsis.

A second object of the invention is a description of antibody inhibitors of Factor XII activation that are useful medicaments for the prophylactic or therapeutic treatment of sepsis. Exemplary antibody would be polyclonal, monoclonal, or recombinant constructs, or fragments derived therefrom having the binding activity of such antibody or antibody fragments.

A third object of the invention is a description of antibody inhibitors of Factor XII activation that are useful medicaments for the prophylactic or therapeutic treatment of sepsis that are characterized in having some, but not necessarily solely the following properties: substantially inhibits the amidolytic activity of Factor XIIa, indicating that it affects the catalytic center of the molecule; reacts with native Factor XII; and is substantially unreactive with Factor XIIa bound to C1 inhibitor.

A fourth object of the invention is a description of methods for administrating inhibitors of Factor XII activation that are useful medicaments for the prophylactic or therapeutic treatment of sepsis.

These, and other objects of the invention, will be more fully understood after a consideration of the following description of the invention.

Figure 1 shows that the antibody, OT-2, binds to native Factor XII in plasma.

Figure 2 shows the effect of mAb OT-2 that inhibits the amidolytic activity of Factor XIIa.

Figure 3 shows that OT-2 inhibits activation of the contact system in plasma. Inhibition occurs at concentrations equimolar to those of Factor XII. DXS stands for dextran sulfate.

Figure 4 shows that mAb OT-2 inhibits activity of Factor XIIa bound to an activator, glass, whereas a natural inhibitor of Factor XIIa, C1 esterase inhibitor (C1-inh.) does not.

Figure 5 shows that the antibody OT-2 does not bind to Factor XIIa-C1-inhibitor complexes.

Several patents/patent applications and scientific references are referred to below that discuss various aspects of the materials and methods used to realize the invention. Because the invention draws on these materials and methods, it is thus intended that all of the references, in their entirety, be incorporated by reference.

The kernel of the instant invention is the realization that Factor XII plays a key role in the onset and progression of sepsis, and that inhibition of its activation would prevent or be useful for treating the disease. The preferred embodiment inhibitors that would have this activity are antibody, or molecules with the binding activity of antibody, but by no means should the invention be constructed so narrowly. Virtually any composition that prevents the activation of Factor XII is intended to come within the scope of the invention.

Exemplary antibody inhibitors of Factor XII activation are preferably but not exclusively polyclonal, monoclonal, recombinant antibody constructs, or fragments derived therefrom having the binding activity of such antibody or antibody fragments. Further, applications of bispecific antibody inhibitors are readily imagined. U.S. Patent No. 4,474,893.

To more clearly define the present invention, particular terms herein will be employed according to the following definitions generally consistent with their usage in the art.

"Sepsis" is herein defined to mean a disease resulting from bacterial infection due to the bacterial endotoxin, lipopolysaccharide (LPS). It can be induced by at least

the six major gram-negative bacilli and these are Pseudomonas aeruginosa, Escherichia coli, Proteus, Klebsiella, Enterobacter and Serratia. It is expected that sepsis induced by gram-positive organisms may also be beneficially treated with the approaches described herein.

"Factor XIIa" refers to activated Factor XII, without intending to denote whether or not the heavy chain is contained within this active species.

"α-Factor XIIa" refers to activated Factor XII, that still contains the heavy chain, this form consists of a disulfide linked dimer (Mr 80,000) of a heavy chain (Mr 50,000) which contains the binding site, and a light chain (Mr 28,000) which contains the catalytic center.

"ß-Factor XIIa" refers to an active form of Factor XII with Mr 30,000, which consists of the complete light chain (with the catalytic center) and a disulfide linked fragment, Mr 2,000, of the original heavy chain. ß-Factor-XIIa is formed upon cleavage of the heavy chain of α-Factor-XIIa.

"Monoclonal antibody" refers to a composition of antibodies produced by a clonal population (or clone) derived through mitosis from a single antibody-producing cell. A composition of monoclonal antibodies is "substantially free of other antibodies" when it is substantially free of antibodies that are not produced by cells from the clonal population. The term "substantially free" means approximately 5% (w/w) or fewer contaminating antibodies in the composition. Also intended to come within the scope of the definition are modifications to antibody that increase its effectiveness. A preferred modification includes conjugation of a water soluble polymer. Preferably the water soluble polymer is polyethylene glycol, or a functionally related molecule such as, for example, polypropylene glycol homopolymers, polyoxyethylated polyols, and polyvinyl alcohol. Derivatization of antibody with such water soluble polymers increases its in vivo half-life, reduces its immunogenicity, and reduces or eliminates aggregation of the protein and may reduce its immunogenicity and aggregation that might occur when it is introduced in vivo. Derivatization of proteins generally, or antibody specifically, with water soluble polymers such as those described above are presented in U. S. Patent Nos. 4,179,337, issued December 18, 1979, to Davis et al., entitled "Non-immunogenic polypeptides"; and 4,732,863, issued March 22, 1988, to Tomasi, et al., entitled "PEG-modified antibody with reduced affinity for cell surface Fc receptors", respectively.

An "antibody-producing cell line" is a clonal population or clone derived through mitosis of a single antibody-producing cell capable of stable growth in vitro for many generations.

"Recombinant antibody" refers to antibody wherein one portion of each of the amino acid sequences of heavy and light chain is homologous to corresponding sequences in antibody derived from a particular species or belonging to a particular class, while the remaining segment of the chains is homologous to corresponding sequences in another. Most commonly, in a recombinant antibody the variable region of both light and heavy chain mirrors the variable regions of antibody derived from one species of mammals, while the constant regions are homologous to the sequences in antibody derived from another. However, this is not necessarily always the case; for example, Ward, et al., 1989, Nature, 341:544, have shown that variable chain alone can be expressed in bacteria with significant antigen binding activity. Also intended to come within the scope of "Recombinant antibody" is monoclonal Fab antibody produced using the techniques described by Huse, W.D. et al., 1989, Science 246:1275.

Two antibodies are "cross-blocking" or have a "shared epitope" when each antibody effectively blocks the binding of the other antibody in a binding inhibition assay. Thus, if antibodies A and B are cross-blocking, antibody A would not bind to its antigen when the antigen had been preincubated with antibody B, and antibody B would not bind to its antigen when the antigen had been preincubated with antibody A.

The term "binding affinity" or "Ka" of an antibody to its epitope, as used herein, refers to a binding affinity calculated according to standard methods by the formula Ka = 8/3(It-Tt), where It is the total molar concentration of inhibitor uptake a 50% tracer, and Tt is the total molar concentration of tracer. See Muller, 1980, J. Immunol. Methods, 34: 345-352.

As used herein, the term "incubation" means contacting antibodies and antigens under conditions that allow for the formation of antigen/antibody complexes (e.g., proper pH, temperature, time, medium, etc.). Also as used herein, "separating" refers to any method, usually washing, of separating a composition from a test support or immobilized antibody, such that any unbound antigen or antibody in the composition are removed and any antigen/antibody complexes on the support remain intact. The selection of the appropriate incubation and separation techniques is within the skill of the art.

By C1 inhibitor is meant a plasma glycoprotein with a molecular weight of about 105,000 that belongs to the super family of serine protease inhibitors. It inhibits activated components of the classical pathway of complement, C1r and C1s, and the intrinsic coagulation system, Factor XIa, Factor XIIa, and Kallikrein. C1 inhibitor also interacts with plasmin and tissue plasminogen activator. C1 inhibitor has the further property of itself being inactivated by proteases, notably elastase. It will, or course, be

understood that intended to come within the scope of the definition of the C1 inhibitor are fragments of the molecule that maintain biologically activity.

In a preferred embodiment of the invention, Factor XII antibody producing immunologic cells are isolated from a mammal immunized with Factor XII, and immortalized to yield antibody secreting cell lines e.g., hybridomas, triomas, quadroma etc. Cell lines that secrete the desired antibody can be identified by assaying culture supernatants for antibody activity. Thus, the invention can be broken down into three sections, and each section discussed separately. That is, the immunization procedure, the cell immortalization procedure, and the identification of the desired Factor XII antibody.

Factor XII produced using a variety of purification schemes may be used to immunize an appropriate host animal. The preferred purification scheme is described by Griffin and Cochrane, 1976, Methods Enzymology, 45:56. The Factor XII so obtained may be affinity purified as described below.

A variety of distinguishable immunization protocols may be employed, and may consist of a primary intravenous, subcutaneous, or intraperitoneal immunization followed by one or more boosts. A suitable adjuvant may be used to enhance the immune response to Factor XII. An exemplary adjuvant is Freund's adjuvant. The precise immunization schedule is generally not critical, and determinative of which procedure is employed, is the presence of Factor XII antibody in the host animal as measured by a suitable assay, described below. A preferred immunization procedure, however, consists of hyperimmunization with Factor XII by repeated intraperitoneal injections as described by Nuijens, J. H., et al., 1989, J. Biol. Chem., 264:12941.

Alternatively, lymphocytes may be immunized in vitro. For example, immunization of peripheral blood cells may be achieved as described by Boss, Methods of Enzymology, 121(l), and in EPA 86106791.6. Note particularly in vitro immunization techniques that can be used to produced either murine or human monoclonal (Procedures for Transforming Cells, pages 18-32, 140-174, Methods of Enzymology, vol.121, part l). Such techniques are also described by Luben, R. and Mohler, M., 1980, Molecular Immunology, 17:635, Reading, C. Methods in Enzymology, 121 (Part One):18, or Voss, B., 1986, Methods in Enzymology, 121:27. A number of in vitro immunization systems have been shown to be effective for sensitizing human B-cells. Reading, C., 1982, J. of Immun. Methods, 53:261.

In lieu of using Factor XII as immunogens, an alternative approach is to synthesize Factor XIIa peptides, and use these as immunogens. For example, preferred peptides would be those that are at the catalytic enter of the molecule, and preferably would encompass amino acid residues his at position 393, asp at position

442 or ser at position 544. The numbering follows the scheme put forth by Cool et al 1985, J. Biol. Chem. 260; 13666. The methods for making antibody to peptides are well known in the art and generally require coupling the peptides to a suitable carrier molecule, such as serum albumin. The peptides can be made by techniques well known in the art, such as, for example, the Merrifield solid-phase method described in Science, 232:341-347 (1985). The procedure may use commercially available synthesizers such as a Biosearch 9500 automated peptide machine, with cleavage of the blocked amino acids being achieved with hydrogen fluoride, and the peptides purified by preparative HPLC using a Waters Delta Prep 3000 instrument, on a 15-20 μm Vydac C4 PrepPAK column. Once clones are identified that secrete anti-peptide antibody, the antibody can be screened for binding and neutralizing activity to Factor XII.

Antibody to Factor XIIa may be either polyclonal, monoclonal, recombinant or fragments derived therefrom. The antibody is preferably human or humanized, although non-human antibody will perform satisfactory.

The preparation of high-titer neutralizing polyclonal antibody can be realized by immunizing a variety of species and employing one of several different immunization regimes. The preferred method of the instant invention is to immunize rabbits with Factor XII prepared in complete Freund's adjuvant by injection into axial lymph nodes. The animals are subsequently subjected to multiple boosts (containing about half the original amount of Factor XII) in incomplete Freunds adjuvant at about 21-day intervals. About 10 days following each 21-day interval, 20-30 ml of blood is removed, the serum isolated and antibody isolated therefrom. This procedure may be carried out for a period of several months.

Monoclonal antibody may be produced using Factor XII, or peptides/peptide conjugates of these molecules as described above, and using the procedures described by Kohler, G. and Milstein, C., 1975, Nature, 256:495, or modifications thereof that are known in the art. Using the screening assays described below, the specificity of antibody produced can be discerned.

The initial work of Kohler and Milstein, above, involved fusing murine lymphocytes and drug selectable plasmacytomas to produce hybridomas. A suitable plasmacytoma is Sp 2/0-Ag14 and is widely used by practitioners of this art. Subsequent to the work of Kohler and Milstein, the hybridoma technique has been applied to produce hybrid cell lines that secrete human monoclonal antibodies. The latter procedures are generally described in Abrams, P., 1986, Methods in Enzymology, 121:107, but other modifications are known to those skilled in the art.

Regardless of whether murine or human antibody is produced, the antibody secreting cells are combined with the fusion partner and the cells fused with a suitable fusing agent, preferably polyethylene glycol, and more preferably polyethylene glycol 1000. The latter is added to a cell pellet containing the antibody secreting cells and the fusion partner in small amounts over a short period of time accompanied with gentle agitation. After the addition of the fusing agent, the cell mixture is washed to remove the fusing agent and any cellular debris, and the cell mixture consisting of fused and unfused cells seeded into appropriate cell culture chambers containing selective growth media. After a period of several weeks, hybrid cells are apparent, and may be identified as to antibody production and subcloned to ensure the availability of a stable hybrid cell line.

The preferred antibody is human monoclonal antibody which can be prepared from lymphocytes sensitized with Factor XII either in vivo or in vitro by immortalization of antibody-producing hybrid cell lines, thereby making available a permanent source of the desired antibody, using the cell fusion techniques described above. Alternatively, sensitized lymphocytes may be immortalized by a combination of two techniques, viral transformation and cell fusion. The preferred combination consist of transforming antibody secreting cells with Epstein-barr virus, and subsequently fusing the transformed cells to a suitable fusion partner. Such fusion partners are known in the art, and exemplary partners may be a mouse myeloma cell line, a heteromyeloma line, or a human myeloma line, or other immortalized cell line. PCT Patent Application No. 81/00957; Schlom et al., 1980, PNAS USA, 77:6841; Croce et al., 1980, Nature, 288:488. The preferred fusion partner is a mouse-human hetero-hybrid, and more preferred is the cell line designated F3B6. This cell line is on deposit with the American Type Culture Collection, Accession No. HB8785. It was deposited April 18, 1985. The procedures for generating F3B6 are described in European Patent Application, Publication No. 174,204.

Techniques applicable to the use of Epstein-Barr virus transformation and the production of immortal antibody secreting cell lines are presented by Roder, J. et al., 1986, Methods in Enzymology, 121:140. Basically, the procedure consist of isolating Epstein-Barr virus from a suitable source, generally an infected cell line, and exposing the target antibody secreting cells to supernatants containing the virus. The cells are washed, and cultured in an appropriate cell culture medium. Subsequently, virally transformed cells present in the cell culture can be identified by the presence of the Epstein-Barr viral nuclear antigen, and transformed antibody secreting cells can be identified using standard methods known in the art.

It will be apparent to those skilled in the art, and as mentioned above, while the preferred embodiment of the instant invention is neutralizing Factor XII monoclonal

antibody, such antibody(s) may be altered and still maintain biological activity. Thus, encompassed within the scope of the invention is antibody modified by reduction to various size fragments, such as $F(ab')_2$, Fab, Fv, or the like. Also, the hybrid cell lines that produce the antibody may be considered to be a source of the DNA that encodes the desired antibody, which may be isolated and transferred to cells by known genetic techniques to produce genetically engineered antibody. An example of the latter would be the production of single chain antibody having the antibody combining site of the hybridomas described herein. Single chain antibody is described in U.S. Patent No. 4,704,692.

A second example of genetically engineered antibody is recombinant, or chimeric antibody. Methods for producing recombinant antibody are shown in U.S. Patent No. 4,816,567, to Cabilly, et al.; Japanese patent application, Serial No. 84169370, filed August 15, 1984; British patent application 8422238, filed on September 3, 1984; and Japanese patent application, No. 85239543, filed October 28, 1985. Also, British patent application, No. 867679, filed March 27, 1986, describes methods for producing an altered antibody in which at least parts of the complementary determining regions (CDRs) in the light or heavy chain variable domains have been replaced by analogous parts of CDRs from an antibody of different specificity. Using the procedures described therein it is feasible to construct recombinant antibody having the CDR region of one species grafted onto antibody from a second species that has its CDR region replaced.

Regardless of the type of antibody, polyclonal, monoclonal or recombinant., it is desirable to purify the antibody by standard techniques as is known in the art, or as described by Springer, 1980, Monoclonal Antibodies.:194, (Eds. Kennett, T. McKearn and K. Bechtol, Plenum Press, New York. Generally, this consists of at least one ammonium sulfate precipitation of the antibody using a 50% ammonium sulfate solution. Antibody affinity columns may also be used.

The preferred Factor XII antibody is denoted OT-2, and methods and procedures for obtaining it are described below in the Example section.

Cell lines that secrete Factor XII antibody can be identified by assaying culture supernatants, ascites fluid etc., for antibody. The preferred screening procedure consists of two sequential steps. First, hybridomas are identified that secrete antibody; and second, the antibody is assayed to determine if it exhibits neutralizing activity. The latter consists of determining whether the antibody blocks the activation of Factor XII.

As applied to cell culture supernatants, the initial screening step is preferably done by RIA or ELISA assay. Both assays are known in the art, and consists of binding Factor XII to a solid matrix, and assaying for antibody binding to Factor XII as

revealed by a second, labelled antibody. If peptides are used as immunogen, the initial screening step determines if the antibody binds to peptide conjugates bound to a solid matrix.

The preferred assay is an ELISA assay as described by Smeenk, R.J.T., et al., 1987, Arthritis Rheum. 30:607. For as description of additional ELISA assay methods see Langone, J. and Van Vinakis, H., 1983, Methods of Enzymology, 92, Part E, and for a description of RIA assay see Miller et al., 1983, Method in Enzym., 121:433 Part I. If peptides are used as immunogen, the initial screening step determines if the antibody binds to peptide conjugates bound to a solid matrix.

An additional assay for Factor XII antibody may be conducted which determines if antibody immunoprecipitates Factor XII from solution. For example, supernatants being tested for the presence of antibody may be incubated with labelled Factor XII for an appropriate time to allow antigen/antibody complexes to form. The complex may be washed to removed any unreacted reagents, and next the antibody complexes incubated with anti-xenotypic or anti-isotypic antibodies specific for the monoclonal antibody being screened. These anti-xenotypic or anti-isotypic antibodies may be immobilized, for example, on a plastic bead. Thus, if Factor XII monoclonal antibody is being screened, then labelled Factor XII will be indirectly bound to the bead and thereby immunoprecipitated. Factor XII antibody can then be quantitated using suitable detection methods known in the art dependent on the nature of the label used. Also, the material can be dissociated from the bead using standard techniques and identified by techniques known in the art, including gel electrophoresis.

The preferred electrophoresis procedure is Western Blot gel analysis as described by Burnette, 1981, Anal. Bio. Chem., 112:195. The Western blots are blocked, washed, and probed preferably in 10 mM sodium phosphate buffer containing 150 mM sodium chloride (pH 7.4), with 0.1% bovine serum albumin (w/v), and 0.1% ovalbumin (w/v). In addition, a detergent is preferably employed such as Tween 20 at a concentration of about 0.1%. Sodium azide may also be included in the solution at a concentration of 0.02%. The blots are preferably first probed with either hybridoma culture supernatant, or dilute ascites fluid containing Factor XII antibody, washed, and then antibody binding revealed with ^{125}I-protein A for about 30-60 minutes. The blots are washed, and subjected to autoradiography using X-ray film.

To expedite the time it takes to assay for Factor XII antibody, several culture supernatants may be combined and assayed simultaneously. If the mixture is positive, then media from each well may subsequently be assayed independently to confirm the presence of antibody.

The antibodies employed in the present invention can be immobilized on any appropriate solid test support by any appropriate technique. The solid test support can be any suitable insoluble carrier material for the binding of antibodies and immunoassays. Many such materials are known in the art, including, but not limited to, nitrocellulose sheets or filters; agarose, resin, plastic (e.g. PVC or polystyrene) latex, or metal beads; plastic vessels; and the like. Many methods of immobilizing antibodies are also known in the art. See, e.g., Silman et al., 1966, Ann. Rev. Biochem., 35: 873; Melrose, 1971, Rev. Pure & App. Chem., 21: 83; Cuatrecaas et al., 1971, Meth. Enzym., 22. Such methods include covalent coupling, direct adsorption, physical entrapment, and attachment to a protein-coated surface. In the latter method, the surface is first coated with a water-insoluble protein such as zein, collagen, fibrinogen, keratin, glutelin, etc. The antibody is attached by simply contacting the protein-coated surface with an aqueous solution of the antibody and allowing it to dry.

Any combination of support and binding technique which leaves the antibody immunoreactive, yet sufficiently immobilizes the antibody so that it can be retained with any bound antigen during a washing, can be employed in the present invention. A preferred solid test support is a plastic bead.

As discussed above, the assay of the present invention employs a labelled antibody. The label can be any type that allows for the detection of the antibody when bound to a support. Generally, the label directly or indirectly results in a signal which is measurable and related to the amount of label present in the sample. For example, directly measurable labels can include radio-labels (e.g. 125I, 35S, 14C, etc.). A preferred directly measurable label is an enzyme, conjugated to the antibody, which produces a color reaction in the presence of the appropriate substrate. (e.g. horseradish peroxidase/o-phenylenediamine). An example of an indirectly measurable label is antibody that has been biotinylated. The presence of this label is measured by contacting it with a solution containing a labeled avidin complex, whereby the avidin becomes bound to the biotinylated antibody. The label associated with the avidin is then measured. A preferred example of an indirect label is the avidin/biotin system employing an enzyme conjugated to avidin, the enzyme producing a color reaction as described above.

Whatever label is selected, it results in a signal which can be measured and is related to the amount of label in a sample. Common signals are radiation levels (when radioisotopes are used), optical density (e.g. when enzyme color reactions are used) and fluorescence (when fluorescent compounds are used). It is preferred to employ a nonradioactive signal, such as optical density (or color intensity) produced by an

enzyme reaction. Numerous enzyme/substrated combinations are known in the immunoassay art which can produce a suitable signal. See, e.g., U.S. Patent Nōs. 4,323,647 and 4,190,496, the disclosures of which are incorporated herein.

The Factor XII antibody described herein, alone or in combination, may be used to passively immunize a host organism suffering from bacteremia or sepsis, or at risk with respect to bacterial infection. Treatment will generally consist of administering the antibodies parenterally, and preferably intravenously. The dose and administration regime will be a function of whether the antibody is being administered therapeutically or prophylactically, and the patient's medical history. Typically, the amount of antibody administered per dose will be in the range of about 0.1 to 25 mg/kg of body weight, with the preferred dose being about 0.1 to 10 mg/kg of patient body weight. For parenteral administration, the antibodies will be formulated in an injectable form combined with a pharmaceutically acceptable parenteral vehicle. Such vehicles are well known in the art and examples include water, saline, Ringer's solution, dextrose solution, and solutions consisting of small amounts of the human serum albumin. The vehicle may contain minor amounts of additives that maintain the isotonicity and stability of the antibody. The preparation of such solutions is within the skill of the art. Typically, the antibodies will be formulated in such vehicles at a concentration of about 2-8.0 mg/ml to about 100 mg/ml.

The effectiveness of the subject Factor XII antibody in the treatment of sepsis can be demonstrated in one of several animal model systems. The preferred animal model system is baboon, and is described by Taylor, et al., 1987, J. of Clinical Inv., 79:918, and by Taylor, et al., 1988, Circulatory Shock, 26:227. Briefly, this consist of infusing a lethal dose of E. coli, about 4×10^{10} organisms per kilogram of body weight administered over a 2-hour period. This is sufficient to kill 100% of the test animals in a period ranging from 16-32 hours. The animals are anesthetized with sodium pentobarbital in the cephalic vein through a percutaneous catheter. They are also orally intubated and positioned on their right side on a heating pad. Blood samples are removed from the femoral vein which is aseptically cannulated in the hind limb. The percutaneous catheter is used to infuse the E. coli organisms. Blood samples are taken at desired time intervals and assayed for white blood cells hematocrit, platelet levels, and fibrinogen. Additionally, mean systemic arterial pressure (MSAP) may be monitored with a transducer (Stratham P2306, Porter) pressure gauge. Changes in these parameters may be prognostic of a patients ability to withstand exposure to a lethal dose of bacteria.

Having described what the applicants believe their invention to be, the following examples are presented to illustrate the invention, and are not to be construed

as limiting the scope of the invention. For example, variation in the source, type, or method of producing antibodies; different labels and/or signals; test supports of different materials and configurations; different immobilization methods may be employed without departing from the scope of the present invention.

Example I

Preparation of Factor XII

Factor XII was prepared by the method described by Griffin and Cochrane, 1976, Methods Enzymology, 45:56, which utilizes ion-exchange chromatography on DEAE-Sephadex and SP-Sephadex.

Affinity purified Factor XII was used in some of the assays described below and was prepared as follows. About 350 ml of citrated plasma pulled from three human donors was made 0.1% (w/v) Tween 20, 10 mM EDTA, 10 mM benzamidine, 0.01% (w/v) STI, 0.05% (w/v) polybrene, and 0.01% NaN_3. The plasma was filtered through a monoclonal antibody column consisting of the monoclonal antibody F3 coupled to Sepharose. The F3 antibody binds to both native and activated Factor XII in the light chain region. The antibody is described by Nuijens et al., 1989, J. Biol. Chem., vol. 264, 12941. The F3-Sepharose affinity column was prepared as described below. Subsequently, the column was washed with 100 ml of PBS containing 0.1% (w/v) Tween 20, 10 mM EDTA, and 0.5 M NaCl. This procedure removes most non-specifically absorbed protein.

Next, Factor XII was eluted from the affinity column with 3 M KSCN in PBS, and the resulting eluate (50 ml) was dialyzed against buffer that consisted of 4 mM sodium acetate, 2 mM acetic acid, 015 M NaCl, 1 mM EDTA, and 0.02% NaN_3, pH 5.2. This procedure has a yield of about 45% of Factor XII. The Factor XII preparation had a specific coagulant activity of 33 U/mg of protein, and displayed no amidolytic towards the chromogenic substrate S2302.

The affinity column was prepared using the antibody F3. The column was prepared using a 50% ammonia sulphate fraction of the antibody coupled to CNBr-activated Sepharose 4B.

By the above purification method Factor XII was determined, using SDS-PAGE, to have a molecular weight of approximately 80,000.

Example II

Peptide Immunogens

Based on the known amino acid sequence of Factor XII, peptides corresponding to neutralizing epitopes of the molecule are synthesized and used as immunogens to produce antibody. Peptides may be synthesized using the solid-phase method, described in detail in Merrifield R.B., 1985, Sci., 232:341-347, on a Biosearch 9500 automated peptide machine, cleaved with hydrogen fluoride, and purified by preparative HPLC using a Waters Delta Prep 3000 instrument, on a 15-20 μm Vydac C4 PrepPAK column. The preferred peptides for producing antibody to Factor XII have the following amino acid sequence:

1. N-cys-trp-val-leu-thr-ala-ala-his-cys-leu-gln-asp-C (his = residue 393).
2. N-phe-ser-pro-val-ser-tyr-gln-his-asp-leu-ala-leu-C (asp = residue 442).
3. N-gly-thr-asp-ala-cys-gln-gly-asp-ser-gly-gly-pro-leu-C (ser = residue 544).

Before using the peptides to make antibody they are conjugated to a suitable carrier molecule to enhance eliciting an antibody response. These procedures are described in U.S. Patent No. 4,762,706, inventors McCormick, et al.. Suitable carriers are keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). The conjugation is achieved via a sulfhydryl group of a cysteine residue that, if necessary, is added to the amino or carboxyl terminal end of the peptides. A heterobifunctional crosslinking reagent, N-maleimido-6-amino caproyl ester of 1-hydroxy-2-nitro-benzene-4-sulfonic acid sodium salt, is prepared by the following procedure.

One molar equivalent (2.24 g) of 4-hydroxy-3-nitro-benzene sulfonic acid sodium salt (HNSA) is mixed together with one molar equivalent (2.06 g) of dicyclohexylcarbodiimide and one molar equivalent (2.10 g) of N-maleimido-6-aminocaproic acid in 25 ml of dimethylformamide (DMF) at room temperature overnight. A white precipitate of dicyclohexyl urea is formed. The precipitate is filtered and 300 ml diethyl ether is added to the mother liquor. After about 10 minutes to 4 hours a gummy solid precipitated from the mother liquor is formed. This solid will contain 58% of active HNSA ester and 42% of free HNSA.

The analysis consists of dissolving a small amount of the precipitate in phosphate buffer at pH 7.0 and measuring the absorbance at 406 nm; this reading provides the amount of unreacted free HNSA which is the contaminating material in the HNSA ester preparation. Addition of very small amounts of concentrated strong base (such as 5N NaOH) instantly hydrolyses the ester formed and a second reading is taken. Subtraction of the first reading from the second yields the amount of ester in the original material. The solid is then dissolved in DMF and placed on a LH20 Sephadex

column and eluted with DMF so that the ester is separated from the contaminating free HNSA. The progress of purification is monitored by thin layer chromatography using eluting solvents of chloroform, acetone and acetic acid (6:3:1 vol/vol). The product is positively identified as mal-sac HNSA ester by its reactivity with amine. The yield of the pure ester is estimated to be approximately 30% of theoretical; the purified material consists of 99% ester.

The ester thus obtained is found to dissolve fully in water and is stable in water for several hours, provided no nucleophiles are added. When placed in 1 N ammonia the ester produces the corresponding amide with a portion hydrolyzed to free acid. The purified ester is found to be stable for extended periods when stored dessicated.

About 0.5 mg of the purified mal-sac HNSA ester is dissolved in 1 ml of distilled water. A 10 µl aliquot of this solution is diluted into 1 ml of 10 mM phosphate buffer at pH 7.0. The absorbance at 406 nm is used to calculate the concentration of free HNSA as described above. When 50 µl of 4.8N sodium hydroxide solution is added to the diluted aliquot of ester and mixed, the absorbance of the solution at 406 nm increases significantly, indicating that the hydroxide nucleophile rapidly hydrolyses the ester to component acid and free HNSA anion.

The difference between the post-base and initial free HNSA concentration represents the concentration of ester. From the actual concentration of ester and protein amino groups the amount of ester to be added to the protein solution to achieve the desired degree of substitution can be calculated.

The purified HNSA ester is then reacted with BSA as follows (the reaction with KLH is similar to this procedure):

A total of 22 mg (20 µmoles) of BSA (of molecular weight 66,296) is dissolved in 2.0 ml of 0.1 M phosphate buffer at pH 7.5 to yield a total amine concentration of 1.0×10^{-2} moles per liter (assuming 59 lysines/BSA molecule). A calculated amount (11 mg, 2.35×10^{-5} moles) of the above-prepared mal-sac HNSA ester (97.7% pure) in powder form is dissolved in 2.0 ml of BSA solution. The reaction is carried out at room temperature. Ten µl aliquots are removed from the solution at timed intervals and are each diluted into 1.0 ml of 0.01 M phosphate buffer at pH 7.0. The spectrum of each diluted aliquot is recorded using a Hewlett-Packard spectrophotometer and the absorbance at 406 nm measured. A total of 50 µl of 4.8N NaOH is then added to each aliquot, each aliquot is mixed and its spectrum retaken, and the absorbance at 406 nm measured.

From the absorbance at 406 nm before and after addition of base the concentration of ester remaining and the percent ester that reacts are determined for the

reaction mixtures. The results show that the reaction rate is essentially linear over a 15-minute period.

After 15 minutes of reaction time, the reaction is stopped by applying the reaction mixture to a PD10 desalting Sephadex G-25 column (Pharmacia, Inc.) equilibrated with 0.1 M phosphate buffer at pH 6.0. It is found that 2.6×10^{-3} moles/l of the ester reacts, and thus 25.9% of the 59 epsilon-amino groups of BSA are presumably substituted. Thus, the product contains 16 mal-sac groups per molecule.

The product of the first reaction, mal-sac-BSA (or mal-sac-KLH), is isolated by applying the reaction mixture to a PD10 desalting Sephadex G-25 column equilibrated with 0.1 M phosphate buffer at pH 6.0. The column is eluted with 0.1 M phosphate buffer in 1.0 ml fractions. The column elution is followed by monitoring the absorbance spectrum, and peak fractions containing the mal-sac BSA are pooled.

The peptides synthesized as described above are added and the pooled mixture is stirred at room temperature overnight. The conjugates are subjected to extensive dialysis against distilled water and lyophilization, and in some cases are analyzed for changes in amino acid composition. These peptide conjugates may be used to immunize animals, or lymphocytes in vitro to produce the desired antibody.

Example III

Immunization with Factor XII or Peptide Immunogens and the Production of Hybridomas

The following describes the immunization of mice with Factor XII with the aim of isolating immunized lymphocytes and producing murine hybridomas. It will be further appreciated that the procedure can be employed to produce antibody against Factor XII peptides, synthesized and conjugated as described above.

Generally, the procedures described in the following references are followed for generating hybridomas. Shulman, et al., 1978, Nature, 276:269; Oi, et al., in Selected Methods in Cellular Immunology, p 351 (Mischell & Schiigi eds. 1980). Foung, et al., 1983, Proc. Nat'l Acad. Sci. USA, 79:7484. Further references include, Gerhard, et al., 1978, Proc. Nat'l Acad. Sci.USA, 75: 1510; Monoclonal Antibodies (R. Kennett, T. McKearn, & K. Bechtol eds. 1980); Schreier et al., 1980, Hybridoma Techniques; Monoclonal Antibodies and T-Cell Hybridomas (G. Hammerling, U. Hammerling, & J. Kearney eds. 1981); Kozbor et al., 1982, Proc. Nat'l Acad. Sci. USA, 79: 6651; Jonak et al., 1983, Hybridoma, 2: 124; Monoclonal Antibodies and Functional Cell Lines (R. Kennett, K. Bechtol, & T. McKearn eds. 1983); Kozbor et al., 1983, Immunology Today, 4:72-79; Shulman et al., 1982, Nature, 276: 269-270;

Oi et al., 1980, Selected Methods and Cellular Immunology, pg. 351-371 (B. Mischell & S. Schiigi Eds.); Foung et al., 1983, Proc. Nat'l Acad. Sci. USA, 79:7484-7488.

The preferred procedure is described by Nuijens J. H., et al., 1989, The Journal of Biological Chemistry, 264:12941. Briefly, Balb/c mice were hyperimmunized with repeated intraperitoneal injections of 25 μg of Factor XII which consisted of a first injection in complete Freund's adjuvant, followed by subsequent boosts at 3, 6, and 9 weeks after the initial injection. Four days after the final boost, spleens were removed from the immunized animals and spleens immunized with non-affinity purified Factor XII prepared as described above. Three days later, spleens from immunized mice were removed and the splenocytes fused to the murine myeloma cell line, SP 2/0Ag14.

The fusion procedure that was followed is described by Kohler & Milstein, 1975, Nature, 256:495, as modified by Fendly et al., in Hybridoma, 6:359 (1987). Briefly, mice were sacrificed and splenocytes teased from immunized spleens, and washed in serum free Dulbecco's Modified Eagles medium. Similarly, SP 2/0Ag14 myeloma cells were washed, and combined with the splenocytes in a 5:1 ratio of spleen cells to myeloma cells. The cell mixture was pelleted, media removed and fusion affected by the addition of 1.0 ml of 40% (v/v) solution of polyethylene glycol 1500 by dropwise addition over 60 seconds at room temperature, followed by a 60-second incubation at 37°C. To the cell suspension with gentle agitation was added 9 ml of Dulbecco's Modified Eagles medium over 5 minutes. Cell clumps in the mixture were gently resuspended, the cells washed to remove any residual PEG and plated at about 2 x 10^5 cells/well in Dulbecco's Modified Eagles medium supplemented with 20% fetal calf serum. After 24 hours, the cells were fed a 2x solution of hypoxanthine and azaserine selection medium. The cells were plated in a total of 20 microtiter plates, which corresponded to 1960 wells. About 9-10 days later, 80% of the wells exhibited good cell growth, and these were screened for antibody to Factor XII. Using this method the hybridoma OT-2 was identified and isolated.

Antibody may be produced in vitro from the hybridoma cell line OT-2 by culturing the cell line in 1 liter roller-bottles in IMDM media supplemented with 2% fetal calf serum, 50 uM 2-mercaptoethanol, and penicillin and streptomycin. The cells are grown to a density of about 10^6/ml, and a week later the supernatants are collected, and concentrated using a hollow fiber device. To purify the antibody on a protein A column (Pharmacia) solid NaCl is added to the concentrate to a final concentration of 3 M. This solution is diluted 1:1 with a solution consisting of 3 M NaCl and 1.5 M glycine, pH 8.9. The protein A column is equilibrated with the latter buffer, and the

concentrate added to the column, the column washed, and antibody eluted off the column with 100 mM sodium citrate buffer, pH 6.0. Those peaks containing antibody are pooled, dialyzed against phosphate buffered saline, and stored until used.

Peripheral blood lymphocytes are isolated from septic patients, and then infected with Epstein-Barr virus and the infected lymphocytes immortalized by fusion to a selectable myeloma cell line, and the hybrid cell lines so generated isolated and characterized as to antibody production. More specifically, mononuclear cells are separated on Ficoll-hypaque (Pharmacia), and monocytes depleted from the mixture by adherence to plastic. Standard laboratory techniques are utilized to effect these procedures. Next, nonadherent cells are enriched for antibody producers by antigen-specific panning. Panning is a technique generally known in the art, and involves incubation of a population of antibody secreting cells on a plastic surface coated with the appropriate antigen, in this instance Factor XII or peptide immunogens derived from Factor XII, and produced as described in Example I. Those cells that express antibody on their surface bind antigen, and consequently adhere to the plastic surface, whereas cells that do not express cell surface antibody, do not adhere and can be removed by washing. Thus, specific antibody secreting cells are enriched for by this technique.

More specifically, 6-well plates (Costar) are coated with 1-20 μg of Factor XII or peptide immunogens per well in phosphate buffered saline at 4°C overnight. The wells are blocked after the overnight incubation period with phosphate buffered saline containing 1% bovine serum albumin for at least 1 hour at 4°C, and subsequently washed with phosphate buffered saline/BSA. Next, 10^7 lymphocytes in 1 ml of PBS/BSA are added to each well of the six well plates. The lymphocytes are allowed to incubate on the plates for 70 minutes, after which any nonadherent cells are removed by aspiration. The adherent cells are incubated with cell culture medium (IMDM, Sigma Chemical Co., St. Louis, Missouri) containing 10% fetal calf serum.

The adherent cells are subjected to Epstein-Barr virus transformation by adding an equal amount of culture media obtained from growing the Epstein-Barr virus infected marmoset cell line, B95-8, or similar cell line, and thus containing the virus, to media bathing the adherent cells. The cells are cultured in this environment at 37°C for 3 hours, and in this way the lymphocytes in the adherent cell population are subjected to Epstein-Barr infection. Following the infection period, the cells are washed and plated onto 96 well microtitre plates at a density of about 10^4 - 10^5 cells/well in IMDM medium, plus 10% fetal calf serum, and 30% conditioned medium. The latter is derived from a lymphoblastoid cell line, preferably JW5. The medium also contains 5×10^{-5} M

2-mercaptoethanol, 50 µg/ml gentamycin sulfate (Sigma), and 600 ng/ml cyclosporine A (Sandimmune, Sandoz, Basel, Switzerland).

After about 14 to 21 days of incubation, cell culture supernatants are combined and screened for the desired antibody binding activity as described below. Positive hybridomas are subcultured at low density, retested for activity, and grown up and fused to the cell line F3B6 using polyethylene glycol and the plate fusion technique known in the art. The latter technique is described by Larrick, J.W., 1985, Human Hybridomas and Monoclonal Antibodies, E.G. Engleman, S.K.H. Foung, J.W., Larrick, and A.A. Raubitschek, (Eds.), Plenum Press, New York, pg 446. The cell line F3B6 is a heteromyeloma that is sensitive to growth in media containing 100 µM hypoxanthine, 5 µg/ml azaserine and 5 µM ouabain. It is on deposit with the American Type Culture Collection with Accession No. HB8785. Finally, the resulting hybrids are again screened to insure that they produce the desired antibody.

Example IV

Properties of OT-2

Binding of native Factor XII to OT-2 was detected by incubating fresh plasma with OT-2 coupled to Sepharose. Bound Factor XII was assessed by a subsequent incubation with polyclonal ^{125}I-anti-Factor XII antibodies. For comparative purposes, binding of the mAb-F3 to Factor XII was also determined. Details of the procedure are described by Nuijens, J.H. et al., 1989, Blood, 72:1704, for the radioimmunoassay procedure performed with the anti-Factor XII mAb-F3. The results are shown in figure 1. It is apparent from the figure that both antibodies bind to Factor XII.

Additionally, binding of native Factor XII to OT-2 may be determined as follows. Microtiter plates were coated overnight at 4°C with native Factor XII purified from plasma by affinity chromatography (Nuijens, J.H. et al., 1989, J. Biol. Chem., 264:12941). The concentration of Factor XII used to coat the plates was 2 µg/ml in phosphate buffered saline (PBS), pH 7.4. The plates were washed and incubated 60 minutes at 37°C with dilutions of mAb OT-2 in PBS containing 0.1% (w/v) Tween 20. After a second wash, plates were incubated for another 60 minutes at 37°C with 1 to 1000 goat-anti-mouse immunoglobulins antibodies conjugated with horseradish peroxidase and diluted in PBS-Tween. After a wash, peroxidase activity was detected as described by Smeenk et al., 1987, Arthr. Rheum., 30:607. Using this assay it was shown that OT-2 binds to Factor XII.

Inhibition of amidolytic activity of ß-Factor XIIa by mAb OT-2. Twenty-five µl of 0.5 µM of ß-Factor XIIa was incubated with 25 µl of 0.5 µM OT-2 or 25 µl of 0.5 µM mAb F3 for 60 minutes at 37°C. Then 100 µl of the chromogenic substrate

S2302 at a concentration of 2 mM was added and the release of pNA was followed by measuring A405 nm at indicated time-points. The results are shown in Figure 2. It is apparent from the data that OT-2 nearly completely inhibits the amidolytic activity of ß-Factor XIIa. In contrast F3, had little or no effect.

Inhibition of contact activation in plasma by mAb OT-2 was shown by measuring a decrease in kallikrein-C1-inhibitor and Factor XII-C1-inhibitor complexes. Activation was initiated by dextran sulfate. For comparative purposes, the effect of mAb-F3 was also determined. Briefly, one volume of fresh plasma, containing 10 mM EDTA, was incubated with one volume of PBS, with or without various concentrations of the monoclonal antibodies for 60 minutes at 37°C. Then, 2 volumes of dextran sulphate (MW 500,000), 100 µg/ml in PBS, were added, and the mixture was incubated for 20 minutes at 37°C. Next, the extent of contact activation in the mixture was assessed by measuring kallikrein-C1-inhibitor and Factor XIIa-C1 inhibitor complexes using the assay described by Nuijens, J.H., et al., 1989, Blood, 72:1404. The results are shown in Figure 3, along with the appropriate controls. It is apparent that contact activation is inhibited by OT-2 as judged by a decrease in both kallikrein-C1-inhibitor and Factor XIIa-C1 complexes. In contrast, little or no inhibition was caused by F3.

A second experiment was conducted which showed that the antibody OT-2 inhibits contact activated Factor XII. The procedure whereby Factor XII is bound to glass is described by Nuijens et al (1989) J. Biol. Chem., vol. 264: p. 12941. The effect of the antibody at different concentrations was determined by measuring a decrease in prekallikrein activation which was demonstrated by a decreased generation of kallikrein-C1-inhibitor complexes in plasma, expressed as mU per ml. For comparative purposes, the effect of the C1 inhibitor was also tested. Figure 4 shows, surprisingly, that OT-2, but not C1 inhibitor inhibits the activity of bound Factor XII.

mAb OT-2 coupled to Sepharose was incubated for 4 hours at room temperature with fresh plasma in which the contact system was, or was not activated by incubation with dextran sulphate at a concentration of 100 µg/ml for 20 minutes at 37°C. The beads were washed with saline, and C1-inhibitor complexes bound to the beads were detected by a subsequent incubation with polyclonal ^{125}I-anti-C1-inhibitor antibodies. The antibody F3 was similarly coupled to Sepharose, and also assayed for Factor XIIa-C1-inhibitor complex binding activity. Details of the procedures have been described for a similar radioimmunoassay with polyclonal antibodies (Nuijens, J.H., et al., 1987, Thromb. Haemost., 58:778). As shown on the right side of Figure 5, OT-2 does not bind to Factor XIIa-C1-inhibitor complexes. In contrast to OT-2, it was

additionally shown that the mAb F3 does bind to Factor XIIa-C1-inhibitor complexes (left side of Figure 5).

Example V

Factor XII Antibody for the Treatment of Sepsis

The effectiveness of the Factor XII antibody, OT-2, in a baboon sepsis model system would be tested essentially as described by Taylor, et al., 1987, J. of Clinical Inv., 79:918, and by Taylor, et al., 1988, Circulatory Shock, 26:227. Briefly, this consists of first measuring Factor XII levels in baboon plasma in response to a lethal dose of E. coli., and secondly, determining if Factor XII antibody is effective in treating sepsis by preventing the death, or prolonging the lives of septic animals. A lethal dose of E. coli consists of approximately 4×10^{10} organisms.

After administration of a lethal dose of E. coli, Factor XII levels start to decrease and Baboons invariably die within 16-32 hours. Taylor, et al., 1987, J. of Clinical Inv., 79:918, and by Taylor, et al., 1988, Circulatory Shock, 26:227.

The effectiveness of the OT-2 monoclonal in preventing the death or prolonging the life of baboons is tested using an administration routine wherein the antibody is delivered in physiological saline at 5.0 mg of antibody per kg of body weight simultaneously with the bacterial challenge. The monoclonal antibody would considerably extend the lifetime of the baboons that receive the dose of antibody and survive for at least 40-60 hours, respectively. Recall that baboons that receive a lethal dose of E. coli invariably die within 16-32 hours.

Deposition of Cultures

The hybridomas used in the above examples, to illustrate the method of the present invention were deposited in and accepted by the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, USA, under the terms of the Budapest Treaty. The deposit dates and the accession numbers are given below:

Culture	Deposit Date	Accession No.
Hybridoma OT-2		
Hybridoma F3		

These deposits were made under the Budapest Treaty and will be maintained and made accessible according to the provisions thereof.

Availability of the deposited cell lines are not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Also, the present invention is not to be considered limited in scope by the deposited hybridomas, since they are intended only to be illustrative of particular aspects of the invention. Any animal cell line (including any hybridoma) which can be used for production of protein according to the methods described in this patent application is considered within the scope of this invention. Further, various modifications of the invention in addition to those shown and described herein apparent to those skilled in the art from the preceding description are considered to fall within the scope of the appended claims.

The present invention has been described with reference to specific embodiments. However, this application is intended to cover those changes and substitutions which may be made by those skilled in the art without departing from the spirit and the scope of the appended claims.

WE CLAIM:

1. A method for treating sepsis in an organism comprising administering to said organism a composition comprising an effective amount of an inhibitor of Factor XII activation.

2. A method as described in claim 1, wherein said inhibitor is an antibody.

3. A method as described in claim 2, wherein said antibody is selected from the group consisting of polyclonal, monoclonal, single chain, bispecific, or recombinant antibody.

4. A method as described in claim 3, wherein said antibody comprises monoclonal antibody.

5. A method as described in claim 4, wherein said antibody comprises OT-2.

6. An inhibitor of Factor XII activation characterized by the following properties:

a) binds to and inactivates Factor XII in plasma;

b) substantially inhibits enzymatic activities of a-Factor XIIa and b-Factor XIIb; and

c) is substantially unreactive with Factor XIIa-C1 complexes.

7. An inhibitor of Factor XII activation as described in claim 6 wherein said inhibitor is an antibody selected from the group consisting of polyclonal, monoclonal, single chain, bispecific, or recombinant antibody.

8. An inhibitor of Factor XII activation as described in claim 6 wherein said inhibitor comprises a monoclonal antibody.

9. An inhibitor of Factor XII activation as described in claim 6 wherein said inhibitor comprises OT-2 monoclonal antibody.

10. An antibody inhibitor of Factor XII activation characterized by the following properties:

a) binds to and inactivates Factor XII in plasma;
b) substantially inhibits enzymatic activities of Factor XIIa and Factor XIIb; and
c) is substantially unreactive with Factor XII-C1-inhibitor complexes.

11. The monoclonal antibody OT-2.

12. The hybridoma that secretes the monoclonal antibody OT-2

13. A monoclonal antibody that cross-blocks the binding activity of OT-2.

14. An antibody that inhibits activation of the contact system in plasma at concentrations about equimolar to those of Factor XII.

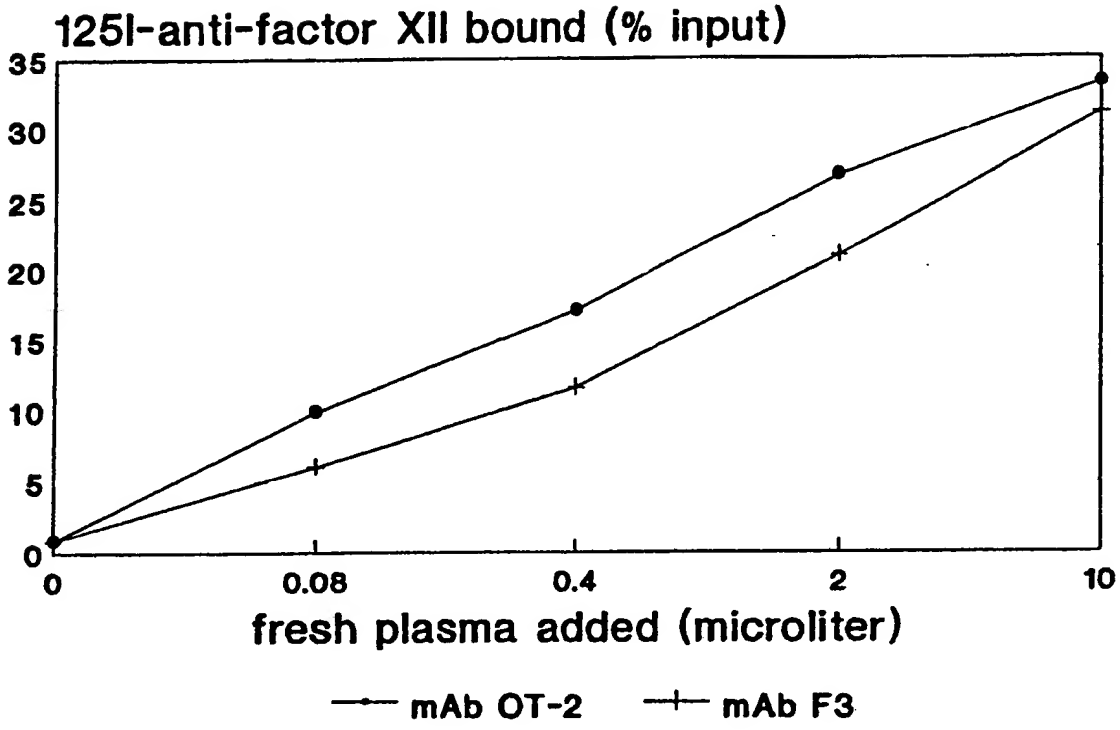
15. An antibody that binds to a peptide selected from the group consisting of N-cys-trp-val-leu-thr-ala-ala-his-cys-leu-gln-asp-C, N-phe-ser-pro-val-ser-tyr-gln-his-asp-leu-ala-leu-C, and N-gly-thr-asp-ala-cys-gln-gly-asp-ser-gly-gly-pro-leu-C.

16. An antibody that binds to a peptide comprising the following amino acids: N-cys-trp-val-leu-thr-ala-ala-his-cys-leu-gln-asp-C.

17. An antibody that binds to a peptide comprising the following amino acids: N-phe-ser-pro-val-ser-tyr-gln-his-asp-leu-ala-leu-C.

18. An antibody that binds to a peptide comprising the following amino acids: N-gly-thr-asp-ala-cys-gln-gly-asp-ser-gly-gly-pro-leu-C.

FIG. 1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35
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25
20
15
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0.08
0.4
2
10
fresh plasma added (microliter)
mAb OT-2
mAb F3

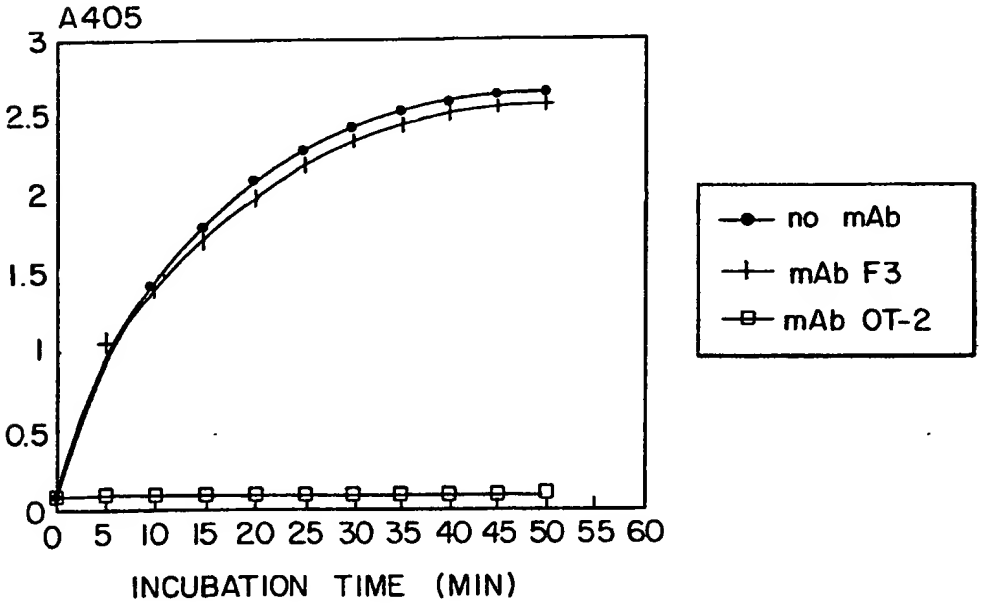
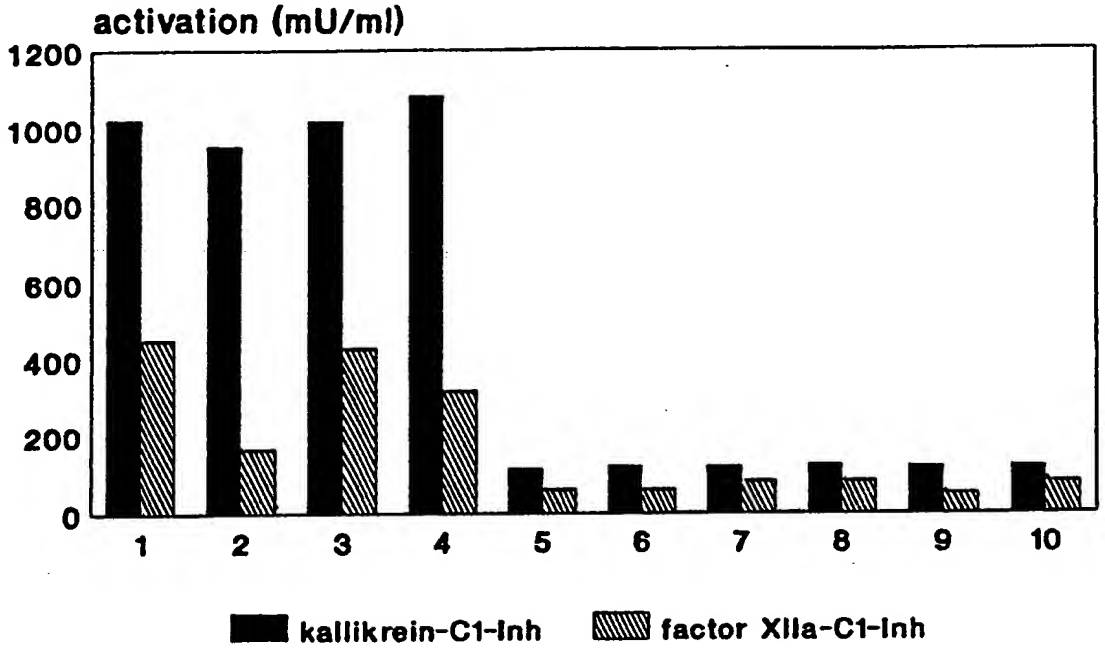

A405
3
2.5
2
1.5
1
0.5
0
0 5 10 15 20 25 30 35 40 45 50 55 60
INCUBATION TIME (MIN)
no mAb
mAb F3
mAb OT-2

FIG. 2

BEST AVAILABLE COPY

FIG. 3



1= Edta-plasma + DXS (100 ugr/ml)

2= „ + „ + mAb F3 (100 µgr/ml)

3= „ + „ + „ (50 µgr/ml)

4= „ + „ + „ (25 µgr/ml)

5= „ + „ + mAb OT-2 (100 µgr/ml)

6= „ + „ + „ (50 µgr/ml)

7= „ + „ + „ (25 µgr/ml)

8= „ + PBS

9= „ + „ + mAb F3 (100µgr/ml)

10= „ + „ + mAb OT-2 („)

FIG. 4

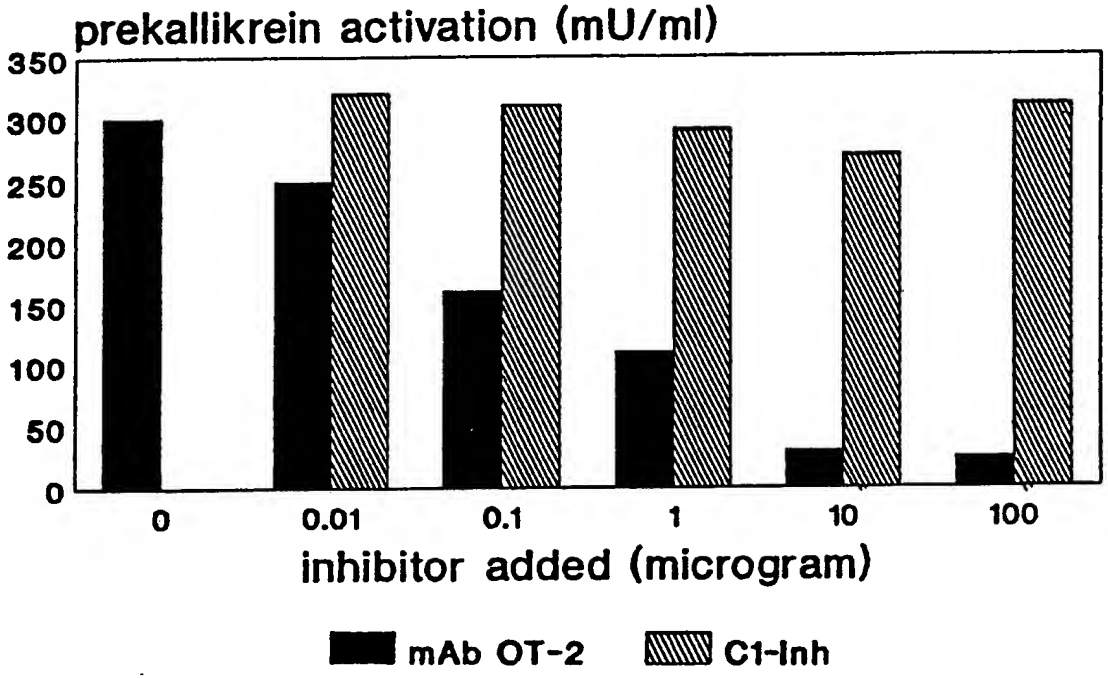
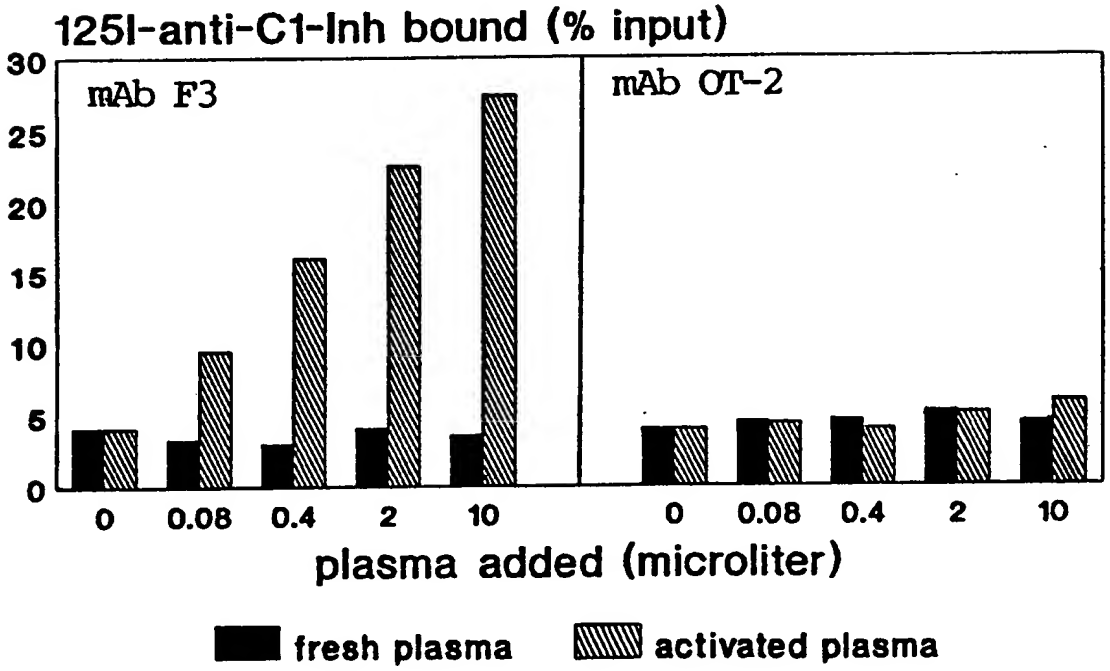


FIG. 5



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/02990

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 12 P 21/08 C 07 K 15/00 A 61 K 39/395 //
C 07 K 7/08

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 K C 12 P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X,P	WO,A,9008835 (COAGEN LTD) 9 August 1990, see page 6, lines 16-20; page 9, lines 13-23; page 13, line 8 - page 20, line 4 ---	6-8,10, 15-18
X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 22, 5 August 1989, The American Society for Biochemistry and Molecular Biology, Inc. (US), J.H. NUIJENS et al.: "Activation of the contact system of coagulation by a monoclonal antibody directed against a neodeterminant in the heavy chain region of human coagulation factor XII (Hageman factor)", pages 12941-12949, see the whole article (cited in the application) ---	6-14
A	WO,A,8911865 (TEMPLE UNIVERSITY OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION) 14 December 1984, see page 10, line 18 - page 14, line 2 (cited in the application) -----	6-8

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
26-07-1991	24 SEP 1991
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Mme N. KUIPER

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 1-5, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv)
Methods for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9102990 SA 47310

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/09/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 9008835	09-08-90	AU-A- 5030590	24-08-90
WO-A- 8911865	14-12-89	US-A- 4963657	16-10-90
		EP-A- 0419574	03-04-91